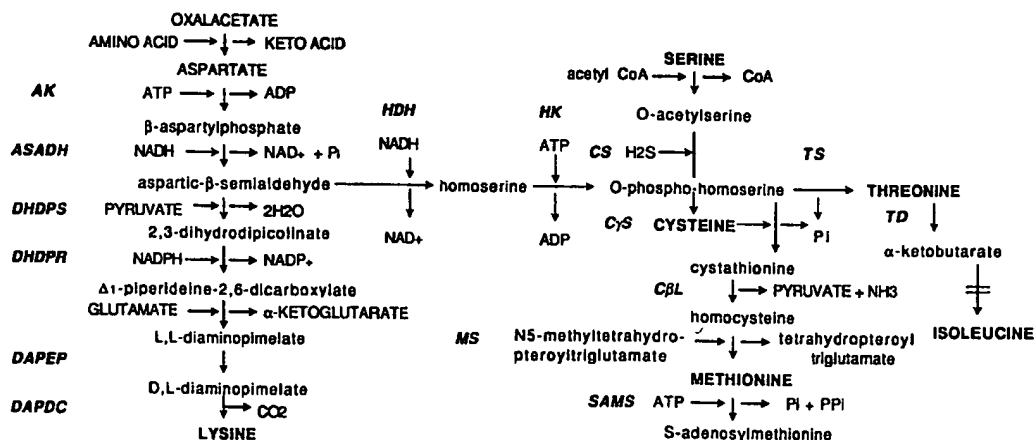




INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification ⁶ : C12N 15/82, 9/02, 9/88, 9/90, 9/10, C12Q 1/68, G01N 33/50, A01H 5/00		A3	(11) International Publication Number: WO 98/55601
			(43) International Publication Date: 10 December 1998 (10.12.98)
(21) International Application Number: PCT/US98/11692		(74) Agent: MAJARIAN, William, R.; E.I. du Pont de Nemours and Company, Legal Patent Records Center, 1007 Market Street, Wilmington, DE 19898 (US).	
(22) International Filing Date: 5 June 1998 (05.06.98)			
(30) Priority Data: 60/048,771 6 June 1997 (06.06.97) US 60/049,443 12 June 1997 (12.06.97) US		(81) Designated States: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GW, HU, ID, IL, IS, JP, KG, KP, KR, KZ, LC, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, RU, SG, SI, SK, SL, TJ, TM, TR, TT, UA, US, UZ, VN, YU, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).	
(71) Applicant (for all designated States except US): E.I. DU PONT DE NEMOURS AND COMPANY [US/US]; 1007 Market Street, Wilmington, DE 19898 (US).			
(72) Inventors; and (75) Inventors/Applicants (for US only): FALCO, Saverio, Carl [US/US]; 1902 Millers Road, Arden, DE 19810 (US). ALLEN, Stephen, M. [US/US]; 12 Stanton Avenue, West Chester, PA 19382 (US). RAFALSKI, J., Antoni [US/US]; 2028 Longcome Drive, Wilmington, DE 19810 (US). HITZ, William, D. [US/US]; 404 Hillside Road, Wilmington, DE 19807 (US). KINNEY, Anthony, John [US/US]; 609 Lore Avenue, Wilmington, DE 19809 (US). ABELL, Lynn, Marie [US/US]; 5 Laurel Court, Wilmington, DE 19808 (US). THORPE, Catherine, Jane [GB/GB]; 120 Ross Street, Cambridge CB1 3BU (GB).			
		Published With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.	
		(88) Date of publication of the international search report: 4 March 1999 (04.03.99)	

(54) Title: PLANT AMINO ACID BIOSYNTHETIC ENZYMES



(57) Abstract

This invention relates to an isolated nucleic acid fragment encoding a plant enzyme that catalyzes steps in the biosynthesis of lysine, threonine, methionine, cysteine and isoleucine from aspartate, the enzyme a member selected from the group consisting of: dihydrodipicolinate reductase, diaminopimelate epimerase, threonine synthase, threonine deaminase and S-adenosylmethionine synthetase. The invention also relates to the construction of a chimeric gene encoding all or a portion of the enzyme, in sense or antisense orientation, wherein expression of the chimeric gene results in production of altered levels of the enzyme in a transformed host cell.

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece	ML	Mali	TR	Turkey
BG	Bulgaria	HU	Hungary	MN	Mongolia	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MR	Mauritania	UA	Ukraine
BR	Brazil	IL	Israel	MW	Malawi	UG	Uganda
BY	Belarus	IS	Iceland	MX	Mexico	US	United States of America
CA	Canada	IT	Italy	NE	Niger	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NL	Netherlands	VN	Viet Nam
CG	Congo	KE	Kenya	NO	Norway	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NZ	New Zealand	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	PL	Poland		
CM	Cameroon	KR	Republic of Korea	PT	Portugal		
CN	China	KZ	Kazakstan	RO	Romania		
CU	Cuba	LC	Saint Lucia	RU	Russian Federation		
CZ	Czech Republic	LI	Liechtenstein	SD	Sudan		
DE	Germany	LK	Sri Lanka	SE	Sweden		
DK	Denmark	LR	Liberia	SG	Singapore		
EE	Estonia						

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/11692

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/82 C12N9/02 C12N9/88 C12N9/90 C12N9/10
C12Q1/68 G01N33/50 A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C12Q G01N A01H

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	FENG, J. ET AL.: "unpublished" EMBL SEQUENCE DATA LIBRARY, 10 May 1997, XP002078204 heidelberg, germany Accession No.B10032	1
A	SAITO, K., ET AL.: "modulation of cysteine biosynthesis in chloroplasts of transgenic tobacco overexpressing cysteine synthase (O-Acetylserine(thiol)-lyase)" PLANT PHYSIOLOGY, vol. 106, 1994, pages 887-895, XP002078205 abstract, page 887, right column; page 888, left column; page 890-894; Fig. 10 --- -/-	1-5, 50-55

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

T later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

X document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

Y document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

G document member of the same patent family

Date of the actual completion of the international search

22 September 1998

Date of mailing of the international search report

15. 01 99

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Holtorf, S

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/11692

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>YOUSSEFIAN, S., ET AL.: "tobacco plants transformed with the O-acetylserine (thiol) lyase gene of wheat are resistant to toxic levels of hydrogen sulphide gas" THE PLANT JOURNAL, vol. 4, no. 5, 1993, pages 759-769, XP002078206 abstract; Fig.1,2,3,5; page 760, left column, last paragraph; pags 764-767 ---</p>	1-5, 50-55
A	<p>BOERJAN, W., ET AL.: "distinct phenotypes generated by overexpression and suppression of S-adenosyl-L-methionine synthetase reveal developmental patterns of gene silencing in tobacco" THE PLANT CELL, vol. 6, October 1994, pages 1401-1414, XP002078207 see the whole document ---</p>	1-5, 50-55
A	<p>US 5 545 545 A (GENGENBACH BURLE G ET AL) 13 August 1996 Fig.1,2; Columns 1,3-7; examples 1,3,5,11 ---</p>	1-5, 50-55
A	<p>US 5 451 516 A (MATTHEWS BENJAMIN F ET AL) 19 September 1995 see the whole document ---</p>	1-5, 50-55
A	<p>WO 96 01905 A (DU PONT ;FALCO SAVERIO CARL (US)) 25 January 1996 pages 1,2,4,7; examples ---</p>	1-5, 50-55
A	<p>WO 96 38574 A (PIONEER HI BRED INT) 5 December 1996 page 4,6,7,8,9; examples, claims ---</p>	1-5, 50-55
A	<p>EP 0 485 970 A (YEDA RES & DEV) 20 May 1992 abstract, column 1-10; examples ---</p>	1-5, 50-55
A	<p>WO 97 07665 A (UNIV HAWAII) 6 March 1997 see the whole document ---</p>	1-5, 50-55
A	<p>CURIEN, G., ET AL.: "characterization of an arabidopsis thaliana cDNA encoding an S-adenosylmethionine sensitive threonine synthase" EMBL SEQUENCE DATA LIBRARY, 26 July 1996, XP002078253 heidelberg, germany cited in the application Accession No. L41666 ---</p>	1-5, 50-55
	<p>--- -/-</p>	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 98/11692

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>ESPARTERO, J., ET AL.: "differential accumulation of S-adenosylmethionine synthetase transcripts in response to salt stress"</p> <p>EMBL SEQUENCE DATA LIBRARY, 23 November 1993, XP002078254 heidelberg, germany cited in the application Accession No. Z24741</p> <p>---</p>	<p>1-5, 50-55</p>
A	<p>SCHWARTZ, D.H., ET AL.: "untitled"</p> <p>EMBL SEQUENCE DATA LIBRARY, 8 June 1996, XP002078255 heidelberg, germany cited in the application Accession No. U49630</p> <p>-----</p>	<p>1-5, 50-55</p>

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 98/11692

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-5 completely, 50-55 partially

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: claims 1-5 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Dihydropicolinate Reductase (DHDPR) from corn and rice; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

2. Claims: claims 6-10 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Diaminopimelate Epimerase (DAPEC) from corn, wheat, rice and soybean; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

3. Claims: Claims 11-15 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Threonine Synthase (TS) from corn; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

4. Claims: Claims 16-20 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Threonine Synthase (TS) from rice; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

5. Claims: Claims 21-25 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Threonine Synthase (TS) from soybean; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

6. Claims: Claims 26-30 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Threonine Synthase (TS) from wheat; recombinant expression of said genes in a transformed host cell; further methods to alter

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

7. Claims: Claims 31-35 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Threonine Deaminase (TD) from corn; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

8. Claims: Claims 36-40 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for Threonine Deaminase (TD) from soybean; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

9. Claims: Claims 41-43 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for S-Adenosylmethionine Synthetase (SAMS) from corn; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

10. Claims: Claims 44-46 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for S-Adenosylmethionine Synthetase (SAMS) from soybean; recombinant expression of said genes in a transformed host cell; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

11. Claims: Claims 47-49 completely; claims 50-55 partially

Isolation of nucleotide sequences coding for S-Adenosylmethionine Synthetase (SAMS) from wheat; recombinant expression of said genes in a transformed host; further methods to alter the level of a chosen amino acid in a plant; to isolate homologous sequences and to screen for inhibitors of said enzymes.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/US 98/11692

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5545545	A	13-08-1996	NONE	
US 5451516	A	19-09-1995	NONE	
WO 9601905	A	25-01-1996	AU 2963695 A BR 9510174 A CA 2192550 A EP 0769061 A HU 77112 A	09-02-1996 04-11-1997 25-01-1996 23-04-1997 02-03-1998
WO 9638574	A	05-12-1996	AU 5956196 A CA 2222673 A EP 0828845 A	18-12-1996 05-12-1996 18-03-1998
EP 0485970	A	20-05-1992	US 5367110 A	22-11-1994
WO 9707665	A	06-03-1997	AU 6899796 A EP 0871356 A	19-03-1997 21-10-1998